

**APPENDIX A**

**"MARKED UP" CLAIMS ILLUSTRATING THE AMENDMENTS MADE TO THE  
CLAIMS OF 09/758,962 WITH ENTRY OF THIS AMENDMENT**

1. A **recombinant plant viral vector comprising a** polynucleotide, **which polynucleotide comprises [comprising]: [(1)] an IRES nucleotide sequence, [(2)] an ORF encoding a peptide of interest, and [(3)] an ORF encoding a viral protein, where the IRES nucleotide sequence[(1)] is located between the peptide of interest ORF [(2)] and the viral protein ORF[(3)].**

2. The **vector**[polynucleotide] according to claim 1 wherein a promoter 5' to **the IRES sequence, the peptide of interest ORF, and the viral protein ORF**[(1), (2) and (3)] **promotes transcription of**[transcribes] a mRNA containing said **polynucleotide** [(1), (2) and (3)].

3. The **vector**[polynucleotide] according to claim 2 wherein the IRES nucleotide sequence is a naturally occurring IRES or a fragment of a naturally occurring IRES that can direct translation of **the peptide of interest ORF** [(2)] or **the viral protein ORF** [(3)].

4. The **vector**[polynucleotide] according to claim 2 wherein the IRES sequence comprises a nucleotide sequence of: SEQ ID NO: 1, [; SEQ ID NO: 2; SEQ ID NO: 3; SEQ ID NO: 4; SEQ ID NO: 5; SEQ ID NO: 6; SEQ ID NO: 7;]or a fragment of SEQ ID NO: 1[, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, or SEQ ID NO: 6, or SEQ ID NO: 7], that can direct translation of **the peptide of interest ORF or the viral protein ORF** [(2) or (3)].

5. The **vector**[polynucleotide] according to claim 2 wherein the viral protein is a coat protein.

6. Cancelled.

7. A recombinant virus comprising a recombinant viral vector according to claim 5.

8. A host comprising a recombinant virus according to claim 7[6].
9. An IRES capable of directing the expression of an internal ORF in a heterologous viral vector.
10. An IRES according to claim 9 wherein the IRES is a IRES<sub>cp</sub>.
11. An IRES according to claim 10 wherein the IRES is crTMV IRES<sub>cp</sub>.
12. A viral vector construct that expresses a bicistronic mRNA comprising an ORF positioned upstream of an IRES sequence and followed by a coat protein coding sequence.
13. A viral vector construct according to claim 12 wherein the ORF encodes a native or foreign gene.
14. A viral vector construct according to claim 53[13] wherein the reporter gene encodes a green fluorescent protein.
15. A viral vector construct, comprising: [(1)] a viral genome, and [(2)] an IRES sequence, wherein the IRES sequence is heterologous to the viral genome, wherein the IRES sequence is downstream of a desired gene or ORF and upstream of a virus coat protein gene, wherein the IRES sequence is in the sense or antisense orientation.
16. A viral vector construct according to claim 15 wherein the viral vector construct expresses a bicistronic mRNA.
17. A viral vector construct according to claim 15 wherein the viral genome is the genome of potato virus X.

18. A potato virus X-based viral vector construct comprising the viral vector construct according to claim 15, wherein the potato virus X-based viral vector construct gives rise to single cell infection sites.

19. A viral vector construct according to claim 15 further comprising [(3)] a stable stem loop structure inserted 5' of the IRES sequence.

20. A viral vector construct according to claim 19 wherein the stem loop structure is immediately upstream of the IRES sequence.

21. A viral vector construct according to claim 20 wherein the stem loop structure causes a reduction in the expression of the virus coat protein gene.

22. A viral vector construct according to claim 21 wherein the stem loop structure interferes with direct interaction of a ribosome at the IRES sequence.

23. A viral vector construct according to claim 15 further comprising [(3)]a stable stem loop structure inserted 3' of the IRES sequence.

24. A viral vector construct according to claim 23 wherein the stem loop structure prevents expression of the virus coat protein gene.

25. A viral vector construct according to claim 23 wherein the stem loop structure effectively blocks scanning ribosomes.

26. A viral vector comprising a **natural or modified plant virus**[plant virus-derived] IRES sequence linked to **an**[the] ORF encoding a protein of interest, wherein **said**[the plant virus-derived] IRES sequence directs translation of the ORF and wherein the protein of interest is heterologous to the viral vector.

27. A viral vector according to claim 26 wherein said[the plant virus-derived]IRES sequence initiates translation effectively in either sense or antisense orientation.

28. A viral vector according to claim 27 wherein said[the plant virus-derived]IRES sequence is an IRES<sub>Scp</sub> sequence.

29. A viral vector construct comprising the function of producing a bicistronic subgenomic RNA in which two ORFs are separated by an IRES.

30. A recombinant plant viral vector construct comprising a modified IRES sequence that directs higher levels of protein expression.

31. Cancelled.

32. Cancelled.

33. Cancelled.

34. Cancelled.

35. Cancelled.

36. Cancelled.

37. Cancelled.

38. A polynucleotide comprising pIRESs-XCP.

39. Cancelled.

40. Cancelled.

41. Cancelled.

42. Cancelled.

43. Cancelled.

44. Cancelled.

45. Cancelled.

46. Cancelled.

47. Cancelled.

48. Cancelled.

49. Cancelled.

50. Cancelled.

51. Cancelled.

52. Cancelled.

53. A viral vector construct according to Claim 12, further comprising a reporter gene.

54. A recombinant or isolated polynucleotide comprising: an IRES nucleotide sequence, an ORF encoding a peptide of interest, and an ORF encoding a viral protein, wherein the IRES nucleotide sequence is located between the peptide of interest ORF and the

**viral protein ORF and wherein the IRES nucleotide sequence is heterologous to the viral protein ORF.**

**55. The polynucleotide of claim 54, wherein one or more of the IRES nucleotide sequence or the viral protein ORF comprises a tobamovirus nucleotide sequence.**

**56. The polynucleotide of claim 55, wherein the tobamovirus comprises crTMV.**